

AD-A259 307



AD _____

2

DETECTION AND CLINICOPATHOLOGIC CORRELATION OF HUMAN
IMMUNODEFICIENCY VIRUS (HIV-1) NUCLEIC ACIDS AND ANTIGENS
IN RETICULOENDOTHELIAL AND CENTRAL NERVOUS SYSTEM TISSUES,
BY IMMUNOHISTOCHEMISTRY, IN SITU HYBRIDIZATION,
AND POLYMERASE CHAIN REACTION

ANNUAL REPORT

ALLEN P. BURKE

SEPTEMBER 30, 1992

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

MIPR 90MM0604

Armed Forces Institute of Pathology
Washington, DC 20306-6000

Approved for public release; distribution unlimited.

The findings in this report are not to be construed as an
official Department of the Army position unless so designated
by other authorized documents

93-00264



1518

93 1 04 259

DTIC
ELECTE
JAN 5 1993
S C D

REPORT DOCUMENTATION PAGE			Form Approved OMB No 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE Sep 30, 1992		3. REPORT TYPE AND DATES COVERED Annual 1 Oct 91 - 30 Sep 92
4. TITLE AND SUBTITLE Detection and Clinicopathologic Correlation of Human Immunodeficiency Virus (HIV-1) Nucleic Acids and Antigens in Reticuloendothelial and Central Nervous System Tissues, by Immunohistochemistry, <u>in situ</u> Hybridization, and Polymerase Chain Reaction			5. FUNDING NUMBERS MIPR 90MM0604 62787A 3M162787A870 AA DA335514	
6. AUTHOR(S) Allen P. Burke				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Armed Forces Institute of Pathology Washington, DC 20306-6000			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research & Development Command Fort Detrick Frederick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words)				
14. SUBJECT TERMS RA 1; HIV-RNA lymphorec-; Ticular; Nervous tissue; Pathology			15. NUMBER OF PAGES	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

CB/N/A citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of this organization.

NA In conduction research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

CB For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

VIA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

William P. Burke
Principal Investigator's Signature Date

Accession For	
NTIS	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Special
A-1	

INTRODUCTION

Nature of Problem. The long-term goal of this work is to establish a laboratory capable of detecting HIV-1 in tissue samples. Cellular localization as well as (semi) quantitation are parts of this goal. These techniques may be useful in staging disease and monitoring therapy. The tissues studied in this proposal will be exclusively from patients with early (i.e., non-symptomatic) HIV disease and will exclude AIDS patients.

Background of previous work. There have been several studies that have identified HIV-1 in tissues by several techniques. Immunohistochemistry and in situ hybridization have been shown to be successful in demonstrating HIV RNA in lymph nodes and central nervous system tissues. The polymerase chain reaction (PCR) has also demonstrated HIV-1 in autopsy as well as surgical tissues from patients with HIV disease. Most of these studies have been performed on tissues from patients with symptomatic HIV disease.

Purpose of present work. The initial goals of this grant project are to collect autopsy tissues from HIV-positive asymptomatic drug addicts who die unexpectedly from drug overdoses. A wide sampling of autopsy tissues will be subjected to various techniques in identifying and localizing HIV-1. The viral burden, cellular sites of replication and progression of viral disease will be studied.

Methods of approach. Three technologies will be employed: immunohistochemistry, in situ hybridization, and polymerase chain reaction. The first two techniques demonstrate viral antigens and nucleic acids, respectively, and provide information on cellular localization. PCR provides a sensitive technique for viral DNA detection and can be modified for semi-quantification of viral burden.

Tissue collection, fixation, and processing. Unsuspected sudden deaths from intravenous drug overdose at the Office of the Chief Medical Examiner (Baltimore, Maryland) were prospectively studied. Cases which were known to be seropositive for HIV-1 before death were excluded. Twelve consecutive seronegative and 25 consecutive seropositive cases were subjected to complete autopsies, including gross and histologic examination of internal organs and toxicologic analysis. The largest lymph nodes from axillary, supraclavicular, mediastinal, inguinal, and mesenteric regions were measured, bisected and one half placed in 10% buffered formalin for 4-12 hours, dehydrated in alcohol and embedded in paraffin for histology, immunohistochemistry and in situ hybridization. 1.5 X 1.5 cm sections of tonsil and spleen were similarly processed.

Determination of HIV-1 infection. At autopsy, post-mortem sera were tested for antibodies to HIV-1 and HIV-2 by immunosorbent assay using the Genie kit (Genetic Systems Corporation, Redmond WA). Seropositivity and seronegativity for HIV-1 was subsequently confirmed by enzyme immuno-linked assay and, if

positive, Western blot testing performed at the Department of Health and Mental Hygiene, State of Maryland.

Immunohistochemistry. For immunohistochemical stains, the avidin-biotin complex method was applied to deparaffinized sections to tissues from all cases. The following antibodies (dilutions in parentheses) were purchased from Dako Corporation (Indianapolis, IN): CD35 (1:20), CD3 (1:250), L26 (1:200), OPD4 (1:50), and HIV p24 (1:25). For CD35, CD3 and p24, tissue sections were predigested with protease K at 37C (0.1 mg/ml) for 20 minutes (Sigma Chemical Co., St. Louis, MO). Biotinylated rabbit or mouse IgG was used as the secondary antibody, and the detection system was Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA). Sections were counterstained with Mayer's hematoxylin.

Histologic staging of lymphoid tissues. Lymphoid patterns were classified as sinus histiocytosis, paracortical expansion, follicular hyperplasia, follicular hyperplasia with fragmentation, follicular involution, and follicular depletion. Staging of lymph node changes in HIV-1 seropositive individuals followed previously published criteria ^{4,5}. Follicular hyperplasia is characterized by numerous germinal centers with prominent mantles and tingible body macrophages. Follicular hyperplasia with fragmentation denotes the presence of enlarged crowded follicles with a depleted and irregular mantle zone; many such lymph nodes have bizarre, fused follicles with a geographic distribution. In this pattern mantles are focally disrupted, some follicular outlines are difficult to ascertain on hematoxylin-eosin stained sections, and follicles are best noted on sections stained with anti-CD35 delineating the follicular dendritic cell network. In follicular involution follicles are small, fragmented, and often scarred, measuring less than 0.8 mm in diameter; there is a lack of tingible body macrophages or mantle zones, and plasma cells are frequent within germinal centers. Follicular depletion indicates a lack of follicles and diffuse infiltration of immunoblasts and plasma cells throughout the lymph node. The degree of plasmacytosis was graded as 1 (clusters of plasma cells in the sinusoidal regions and paracortical areas), 2 (large aggregates of plasma cells filling an entire high-powered 60X field in expanded paracortical and sinusoidal areas), and 3 (sheets of plasma cells effacing the entire lymphoid tissue).

In situ hybridization. The protocol of Cecil Fox ³ was followed with minor modifications. A full-length sheared HIV-1 RNA genomic probe was prepared by transcription and labelled with ³⁵-S labelled CTP (Lofstrand Laboratories). Both sense and antisense probes were prepared using the Promega-Biotech (Madison, WI) RNA synthesis kit. Tissue sections mounted on silanated glass slides were deparaffinized with xylene, hydrated and washed in 0.2 N HCl and digested in protease k (0.1mg/ml). Slides were acetylated with acetic anhydride and

prehybridized for 2 hours at 45C in a solution prepared from 60 ml 5M NaCl, 10 ml 1.0 M Tris-HCl pH 7.4, 1 ml 0.5 M EDTA, 20 ml bovine serum albumin 5%, 2 ml Ficoll, 2 ml polyvinylpyrrolidone (PVP), 5 ml 1 M dithiothreitol (DTT), 25 ml transfer RNA (200 mg/100ml), 375 μ l deionized water, and 500 ml deionized formamide. Hybridization was accomplished in a probe cocktail of 240 μ l 5 M NaCl, 40 μ l 1 M Tris pH 7.4, 4 μ l 0.5 M EDTA, 8 μ l 10% Ficoll, 8 μ l 10% PVP, 100 μ l 2 mg/ml yeast transfer RNA, 20 μ l 1 M DTT, and 80 μ l bovine serum albumin 50 mg/ml). 0.313 ml of hybridization cocktail was added to 1.25 ml 20% dextran sulfate in formamide, 0.737 ml diethyl pyrocarbonate treated distilled water, and 0.2 ml probe containing 200 million dpm. After boiling and ice quenching, probe was distributed on the tissue sections, coverslipped, and incubated overnight in a humid chamber at 45C. Slides were decoverslipped in 2XSSC (NaCl/Na Citrate, pH7.4), washed in 50% formamide and 50% 2X SSC, and then in successive washes of 2X SSC and 0.1X SSC at 60C, digested in RNase A (40 mg/l at 37C for 40 min), dehydrated in 0.3 M ammonium acetate in 95% ethanol, and dried. Slides were exposed in the dark after dipping in Kodak NTB-2 emulsion for 14 days and counterstained in hematoxylin-eosin.

Positive control tissue sections consisted of xenotransplanted lymphoblastoid tumors grown in nude mice (obtained from Neal Wetherall, Biomed Laboratories, Minnetonka, MN) infected with HIV-1 and fixed in a similar manner to test tissues; HIV-1 infected T-cell cytologic preparations (Oncor laboratories, Rockville, MD); HIV-1 infected H9 cells mixed in a 1:100 dilution with non-infected H9 cells; fixed surgical lymph node specimens from three HIV-1 infected patients obtained for diagnostic purposes; and autopsy lymph nodes from a violent death victim with AIDS-related complex. Negative controls included slides incubated with Coxsackievirus B3 specific probes prepared with the Promega-Biotech RNA synthesis kit, slides incubated with sense HIV-1 probe, slides pre-digested with RNase, and slides pre-hybridized with unlabelled probe; all were hybridized similarly to test slides with antisense HIV-1 probe. Negative tissue controls consisted of lymph nodes collected from seronegative drug addicts. Internal controls for the presence of RNA within tissue sections consisted of antisense RNA probes for beta-actin (Lofstrand Laboratories).

Double labelling in situ hybridization and immunohistochemistry. For slides subsequently hybridized with radiolabelled RNA probes, 2X blocking serum and primary antibody were mixed in equal concentrations with RNasin (0.5 μ l/20 μ l) and DTT (0.1M, 0.2 μ l/20 μ l) before application to slides. The remainder of the in situ procedure was identical except that one week was used for exposure, and that hematoxylin only was used as a counterstain.

Results

Sex and age data. Twelve seronegative controls (10 men, 2 women) had a mean age of 35.2 (+/- 7.6) years. 25 seropositive cases (20 men, 5 women, mean age 34.9 +/- 6.5 years) were divided by histologic changes (see below) into a group of follicular hyperplasia (n=14, mean age 32.1 +/- 4.3); and follicular involution (n=11, mean age 39.5 +/- 6.6). The difference in age between follicular hyperplasia and follicular

involution groups was significant ($p=0.003$, Student t-test).

Autopsy data. None of the 37 total cases demonstrated palpable adenopathy, significant pathologic findings or evidence of infection in major viscera; the cause of death in each was attributed to drug overdose. For all lymphoid groups, the size of the largest node was significantly greater in early stage HIV-1 seropositive nodes than controls (Student t-test, p values <0.05), with the exception of mediastinal lymph nodes. Late stage lymph nodes were similar in size to control tissues with the exception of mesenteric and supraclavicular nodes ($p<0.05$).

Lymphoid pathology. Lymphoid tissues of 14 of 25 seropositive cases demonstrated primarily follicular hyperplasia, with or without fragmentation; these were designated as early stage cases. Less common lymphoid patterns present in these early stage cases were follicular involution and normal reactive patterns of sinus histiocytosis with grade 0 or 1 plasmacytosis; follicular depletion was never present. The lymphoid tissues of the remaining 11 seropositive cases demonstrated predominantly follicular involution or depletion; these were designated as late stage cases. Lymphoid tissues demonstrating follicular involution were characterized by varying degrees of paracortical hyperplasia, interfollicular plasma cells and plasma cells within scarred follicles. Follicular depletion was characterized by grade 3 plasmacytosis and varying degrees of sinus histiocytosis. The concordance of lymphoid patterns among lymphoid groups ranged from 3/6 to 6/6 and averaged 69% for early stage and 72% for late stage cases.

Follicular hyperplasia was more prevalent in seropositive lymphoid tissues than seronegative ones, for early and late stages combined ($p=0.02$) or compared to early stage alone ($p=0.00001$). Plasmacytosis of grades 2 or 3 was more prevalent in seropositive lymphoid tissues than seronegative cases, for early and late stages combined ($p<0.0001$), and for early ($p<0.0001$) and late ($p<0.0001$) stage lymphoid tissues compared separately. The mean number of follicles was greater in seropositive lymphoid tissues with follicular hyperplasia than seronegative cases ($p<0.001$), and the mean size of follicles was greater in seropositive cases with follicular hyperplasia or fragmentation, ($p<0.001$). For a given lymphoid tissue, the percentage of follicles containing detectable HIV-1 RNA progressively increased from 49% in follicular hyperplasia to 62% in follicular hyperplasia with fragmentation and 65% in follicular involution.

Follicular hyperplasia was present in 27% of seronegative lymphoid tissues, sinus histiocytosis in 40%, paracortical expansion in 16%, and unstimulated lymphoid tissues in 16%. Those cases that demonstrated follicular hyperplasia did not demonstrate the features characteristic of seropositive cases, namely, loss of mantle zones, follicular crowding with irregularity and fusion, and follicular involution. In general, follicles were small, regular, and peripheral. 39% lacked plasmacytosis, 13% showed grade 1 plasmacytosis, and 6% grade 2 plasmacytosis. None showed grade 3 plasmacytosis. Tonsillar tissues all demonstrated follicular hyperplasia. There was follicular irregularity in 4 tonsillar tissues but the geographic distribution and loss of mantle zones characteristic of HIV-1 infection was absent.

Giant cells were absent in lymphoid tissues of all seronegative individuals. Warthin-Finkeldey giant cells were present in 10 early stage cases and 3 late stage cases in a total of 27 lymphoid tissues. In situ hybridization. Positive controls. Infected xenotransplanted lymphoblastoid cell lines, diluted infected H9 cells, and cytopins demonstrated positivity in a subpopulation of cells in an expected distribution with the HIV-1 antisense probe. Surgically removed lymph node tissues from two HIV-1 seropositive patients demonstrated positive staining in follicular centers as well as interfollicular positive cells in a distribution similar to that reported by Spiegel et al ². Autopsy lymph nodes from an HIV-1 seropositive prostitute with generalized lymphadenopathy demonstrated diffuse signal in follicular centers, which demonstrated irregular outlines. In the above tissues, there was no positive signal using HIV-1 sense probes, HIV-1 antisense probes with pre-digestion with RNase or preincubation with non-labelled antisense probe, or Coxsackievirus B3 antisense probe.

Internal control, antisense beta-actin probe. In tissues from seropositive cases, a majority of tissues demonstrated signal in arterial and arteriolar media, tonsillar epithelium, and germinal centers, indicating the presence of intact messenger RNA. In 15 of 136 tissues, however, technical artifact, possible related to post-mortem changes, precluded accurate evaluation of in situ hybridization results.

Negative controls. None of the 12 seronegative control tissues demonstrated specific signal in follicular centers, interfollicular lymphocytes, or other cells using antisense HIV-1 probe. With the sense HIV-1 probe there was non-specific binding similar to that seen in seropositive cases in pigmented macrophages.

Seropositive cases. Control probes (sense HIV-1, Coxsackievirus B3) demonstrated non-specific binding within pigmented macrophages in scattered interfollicular cells in three inguinal lymph nodes with dermatopathic lymphadenopathy. Rare eosinophils demonstrated showed non-specific binding with negative control probes. Non-specific binding was generally minimal and could easily be distinguished from specific binding by pattern of staining and similar distribution in control and test slides. Mediastinal nodes demonstrated anthracotic pigment, which could be distinguished by its irregular outlines from the perfectly round silver grains of similar black color indicating positive signal. None of the lymph nodes demonstrated signal in follicular centers or over interfollicular lymphoid cells using control probes.

With antisense HIV-1 RNA probe, the predominant pattern of signal was a positive signal over the germinal centers. In lymphoid tissues demonstrating follicular hyperplasia the signal was quite strong and diffuse over the follicular center, occasionally with a peripheral pattern. Signal was usually of weaker strength in lymphoid tissues with hyperplasia with fragmentation or follicular involution. Small fragmented follicles with hyalinization also demonstrated signal. Positive signal was not noted in lymphoid tissues with sinus histiocytosis or follicular atrophy. Serial sectioning demonstrated a pattern identical to that of CD35 staining in all positive cases.

Tonsil and inguinal nodes most often demonstrated positive signal, and spleen least often. Positive signal was present in a high percentage of lymphoid tissues with follicular hyperplasia, hyperplasia with fragmentation, or follicular involution. The high percentage of positive tonsillar and inguinal nodal tissues reflected the increased prevalence of hyperplastic or involuting follicles within these tissues. The extent of positivity, as measured by percentage of total follicles positive combined from all lymphoid tissues examined, was highest in tissues demonstrating follicular involution.

Four tonsils and two inguinal lymph nodes, and one axillary lymph node with follicular hyperplasia demonstrated, in addition to diffuse follicular center staining, scattered interfollicular cells with intense staining. Such cells were not seen in any nodes with follicular involution or follicular atrophy. Fourteen of 24 lymphoid tissues with syncytial cells demonstrated positive signal but it was not localized over syncytial cells in any case.

Immunohistochemistry. Seven of 14 early stage and 3 of 11 late stage cases demonstrated positivity for p24 antigen in at least one lymphoid tissue (range, 1-5). Positivity was limited in all cases to germinal centers and was in a pattern mimicking that of staining with anti-CD35. Lymphoid tissues with regular follicular hyperplasia were more likely positive than those with irregular hyperplasia; lymphoid tissues showing follicular involution showed the least likelihood of positivity and those showing follicular atrophy were all negative. The proportion of positive lymphoid tissues ranged from 12 to 27%. Seven of 24 lymphoid tissues with giant cells demonstrated positivity with p24 antibody but in no case was positivity localized over syncytial cells. Staining of lymphoid tissues containing giant cells revealed that they were not immunoreactive in any case with anti-L26, anti-CD3, anti-LCA, and anti-OPD4. In three lymphoid tissues containing giant cells, there were occasional cells focally positive for KP-1.

Double labelling immunohistochemistry/in situ hybridization

Colocalization of immunohistochemical staining and in situ hybridization signal for antisense HIV-1 probe was present using the CD35 antibody in a follicular center cell distribution.

Interfollicular cells demonstrating in situ hybridization signal for antisense HIV-1 probe did not colocalize with antibody staining for anti-CD3, anti-CD35, anti-KP1, anti-OPD4, or anti-LCA, despite strong labelling of both immunohistochemical staining and in situ signal.

CONCLUSIONS

The aim of this study was to determine the extent of histopathologic changes and presence of HIV-1 viral RNA throughout the lymphoid system in early HIV-1 disease. We found that a high percentage of lymphoid tissues demonstrate follicular hyperplasia with fragmentation and follicular involution characteristic of HIV-1 infection. Follicular fragmentation, involution or depletion are generally present throughout the lymphoid system and there is good concordance from one lymphoid site to another. Therefore, sampling of one lymph node group may be indicative of overall histopathologic pattern. Our data do not indicate that early HIV-1 related

histopathologic changes are concentrated in certain lymphoid sites but suggest that HIV-1 infection is multicentric from an early stage of infection.

The histopathologic changes of HIV-1 infection are not specific, but certain features are suggestive of HIV-1 disease and were not present in our control samples. Warthin Finckeldey syncytial cells, which are present in hyperplastic or atrophic nodes, were absent in non-infected tissues. Hyperplastic follicles with a marked degree of fragmentation, loss of mantles, and irregularity with a geographic pattern, were also absent in control tissues. Lymphoid tissues with involuted or depleted follicles are similar in histologic appearance to quiescent nodes from seronegative controls, but generally demonstrate a much higher degree of plasmacytosis in medullary areas. Although we have demonstrated that lymphoid changes are fairly distinct from a group of non-infected individuals without evidence of lymphoid disease, we did not compare HIV-1 related histopathologic changes to other infectious processes involving the lymphoid system. The specificity of HIV-1 induced changes can only be discussed in the context of a particular patient population.

The clinical stages of HIV-1 disease includes a very early transitory stage of lymphadenopathy (AIDS-related complex)⁸. Because none of our cases had palpable lymphadenopathy, and because seropositive drug addicts would be expected to overdose at all stages of early HIV-1 disease, it is likely that the stage of AIDS-related complex is of short duration in comparison to the clinically latent stage before significant immunodeficiency. With the exception of mediastinal nodes, early stage lymph nodes in our study were slightly but significantly larger than seronegative controls. These data indicate that there is a prolonged stage of mild adenopathy in early HIV-1 disease. The histopathologic patterns of follicular hyperplasia and fragmentation, as have been described in patients with clinically enlarged nodes and AIDS-related complex, are found in this phase of mild adenopathy as well.

There are few data regarding in situ hybridization results in non-enlarged lymph nodes from HIV-1 infected individuals, or from several lymphoid groups from the same HIV-1 infected individual. Previous reports of in situ hybridization have predominantly utilized surgically acquired single lymph nodes from a highly selected population with lymphadenopathy^{1,2,3,6}. It has been recently emphasized that the events occurring in lymph nodes in HIV-1 infected individuals without peripheral lymphadenopathy are unknown³. In such individuals, we have found HIV-1 RNA by in situ hybridization in diverse lymphoid tissues when follicles containing follicular dendritic cells were present. Certain lymphoid sites, such as tonsils and inguinal nodes, were likely to contain HIV-1 RNA, because of a high prevalence of reactive or involuting follicles in these tissues. HIV-1 RNA was also present in splenic tissues demonstrating follicular hyperplasia, a finding not previously reported. The three stages of HIV-1 related lymphoid disease with CD-35 positive germinal centers, namely follicular hyperplasia, follicular hyperplasia with fragmentation, and follicular involution, had HIV-1 RNA within germinal centers in a follicular center cell distribution. This distribution was demonstrated to correspond to the follicular dendritic cell network by comparison with serial sections stained with anti-CD35. The signal

is typically extracellular, as has been emphasized by Fox et al ³, corresponding to follicular dendritic cell processes.

In our tissues, the strength of this signal decreased with the later patterns of lymph node changes, namely follicular fragmentation and lysis, as has been described by Fox et al ³. In later stage lymph nodes with involuted follicles, Fox et al ³ found a lack of staining in a follicular dendritic cell pattern with few positive macrophages within fragmented germinal centers. In contrast to these findings, we found a persistent weak signal in involuted, fragmented follicles in a follicular center cell distribution. In addition, the percentage of positive follicles increased with later patterns of follicular involution. Our data suggest that there may be a progressive recruitment of lymph nodes infected with HIV-1 until the stage of follicular involution, in which HIV-1 RNA is decreased due to gradual destruction of the follicular dendritic network. Similar to other observers ¹⁻³, we did not note positive signal in nodes with follicular depletion.

The significance of HIV-1 RNA in a follicular dendritic cell distribution is debated. It has been postulated, based on *in situ* hybridization, ultrastructural and tissue culture data ^{2,6}, that the follicular dendritic cell is a reservoir of replicating virus which infects circulating CD4 positive T cells. Alternatively, virus trapped as antigen-antibody complexes on follicular dendritic cell processes may continually infect circulating CD4-positive T-cells ^{1,3}. It is not known if the follicular dendritic cell network is destroyed by virus replicating within it, or if it regresses in later stages of disease because of its dependence on circulating CD4 positive T cells for its functional integrity. Studies using polymerase chain reaction *in situ* hybridization for integrated provirus or RNA *in situ* hybridization using probes specific for spliced viral mRNA are necessary to resolve this question.

In addition to diffuse follicular dendritic cell staining, we also demonstrated signal over lymphocytes within germinal centers and interfollicular areas. Scattered intra- and interfollicular cells demonstrating signal for HIV-1 RNA have been demonstrated in the absence of follicular dendritic cell staining ^{9,10}, have been shown to label more strongly than follicular dendritic cells ¹, and have been seen in all histologic patterns of HIV-1 infection ⁹. In double labelling with immunohistochemistry, these cells have been found to represent CD4 positive T-cells ^{2,6}. We found them only in early stages of follicular hyperplasia, and, like Fox et al ³ were unable to corroborate their identity by double labelling immunohistochemistry - *in situ* hybridization ³. If these cells truly represent circulating helper T-cells that express HIV-1, they appear to be numerous only in early stages of follicular hyperplasia and are depleted at the stages of follicular involution and depletion. This finding may have relevance in staging of lymphoid tissues in HIV-1 disease and needs to be correlated with the level of expression of HIV-1 RNA in circulating T-cells, which is believed to be very low, on the order of 1:1000 cells ^{11,12}.

This study demonstrates for the first time that HIV-1 RNA is readily detectable in routinely processed, formalin-fixed paraffin

embedded post-mortem tissues. Because this was a prospective study, the parameters of fixation were carefully controlled, and our results may not be transferrable to archival tissues in which the type and length of fixation is unknown.

In conclusion, our studies suggest that generalized mild lymphadenopathy persist in HIV-1 infected person with early stage disease. There appears to be an association between localization of viral RNA and morphology, in that HIV-1 RNA is detectable in progressively increasing numbers of lymphoid follicles as they undergo hyperplasia, fragmentation and involution. Prospective surgical biopsy studies in well-characterized HIV patients are clearly required to establish the clinical validity of these observations. Similar studies in populations in which different strains of virus are prevalent are also needed to determine if genetic variability is important in tissue distribution and histopathologic effects. In this population, we have demonstrated that there is an extensive load of HIV-1 RNA associated with the follicular dendritic cells throughout the lymphoid system from early stages of infection, and that measurable numbers of lymphocytes with replicating virus are present within lymphoid tissues only in the earliest stages of lymphoid hyperplasia.

TABLE 1

Histopathologic changes and positivity for HIV-1 RNA

Seropositive, early stage

histologic pattern, positivity for HIV-1 RNA

<u>case #</u>	<u>age</u>	<u>tonsil</u>	<u>ax</u>	<u>scl</u>	<u>med</u>	<u>mes</u>	<u>ing</u>
	<u>race</u>						
	<u>sex</u>						
2	34BM	inad	SH -	FF +	SH -	SH -	FF +
6	32BF	FH +	FF +	inad	SH	inad	FI +
12	40BM	FF +	FI +	FI +	FF +	FF +	FF +
19	31BM	inad	FF +	FI +	FF	FF	FF +
21	35BM	FF +	FF -	FI -	FI -	FI -	FI -
22	37BF	inad	FF +	FI -	FI +	FF +	FI +
24	28BM	FI +	FF	FF +	FI	FF	FF +
25	31BM	FF +	FF -	FF -	FI -	FI -	FF +
27	30CM	inad	FF +	FF +	FF +	FI +	FF +
28	29BM	FF +	FF +	FF +	FI +	FF +	FF +
31	24BM	FH +	FF +	FF	FF +	FH -	FF +
32	32BM	FH +	inad	inad	FI -	FH -	FI +
36	29BM	FH +	FF +	FF +	FI +	FI +	FH +
37	38BM	FF -	FF +	FF +	FI +	inad	FF +

Abbreviations: tons = tonsil, ax = axillary, scl = supraclavicular, med = mediastinal, mes = mesenteric, ing = inguinal, inad = inadequate tissue at collection due to lack of lymphoid tissue, + = positive, - = negative.

Blanks indicate lack of beta actin control staining, or tissue artifact

precluding evaluation. FH = follicular hyperplasia, FF = follicular

hyperplasia, with fragmentation, FI = follicular involution; FD = follicular

depletion, B = black, C = Caucasian, F = female

TABLE 2

Histologic pattern, positivity for HIV-1 RNASeropositive cases, late stage

<u>case #</u>	<u>age</u>	<u>tonsil</u>	<u>ax</u>	<u>scl</u>	<u>med</u>	<u>mes</u>	<u>ing</u>
	<u>race</u>						
	<u>sex</u>						
3	51BM	inad	FI +	FI	FI +	inad	FF -
13	32BM	FI +	FF +	FI +	SH	FI +	FI +
17	35BM	FI -	FI	FD	FI	inad	FI +
20	49BF	FF +	FI	FI	FI +	SH	FI +
23	43BM	FF -	FF -	SH -	FD -	FI -	FI -
26	42BM	inad	FD -	FD -	FD -	FD -	FD -
29	35BM	inad	FI -	FI +	FF +	FI +	FI -
30	30BF	FF +	FI +	FI -	FF +	inad	FI +
34	37BM	FI +	FI +	FD -	FI +	FI +	FI +
35	40BM	FI +	FI +	FI +	FI +	inad	FF +
38	40BM	FD -	FD -	FD -	FI -	FI -	FD -

Abbreviations: See table 1

REFERENCES

1. Biberfeld P, Chayt KJ, Marselle IM, Biberfeld G, Gallo RC, Harper ME. HTLV-III expression in infected lymph nodes and relevance to pathogenesis of lymphadenopathy. *Am J Pathol* 125:436-442, 1986.
2. Spiegel H, Herbst H, Niedobitek G, Foss H-D, Stein H. Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T-helper cells. *Am J Pathol* 140: 15-22, 1992.
3. Fox CH, Tenner-Racz K, Racz P, Firpo A, Pizzo PA, Fauci AS. Lymphoid germinal centers are reservoirs of human immunodeficiency virus type 1 RNA. *J Infect Dis* 1991;164:1051-7.
4. Biberfeld P, Ost A, Prowit A, Sandstedt B, Pallesen G, Boettiger B, Morfelt-Mansson L, Biberfeld G. Histopathology and immunohistology of HTLV-III/LAV related lymphadenopathy and AIDS. *Acta Pathol Microbiol Immunol Scand (A)* 95:47-65, 1987.
5. Ost A, Baroni CD, Biberfeld P, Diebold J, Moragas A, Noel H, Pallesen G, Racz P, Schipper M, Tenner-Racz K, van den Tweel JG. Lymphadenopathy in HIV infection: histological classification and staging. *Acta Pathol Microbiol Immunol Scand Suppl* 8:7-15, 1989.
6. Stein H, Spiegel H, Herbst H, Niedobitek G, Foss H-D. Das lymphatische Gewebe und AIDS: Die Rolle der Lymphozyten und der follikulaeren dendritischen Zellen (FDC). *Verh Dtsch Ges Path* 75: 4-19, 1991.
7. Madea B, Roewert HJ, Krueger RF, Ablashi DV, Josephs SF. Search for early lesions following human immunodeficiency virus type 1 infection. A study of six individuals who died a violent death after seroconversion. *Arch Pathol Lab Med* 114:379-382, 1990.
8. Redfield RR, Wright DC, Tramont EC. The Walter Reed staging classification for HTLV-III/LAV infection. *N Engl J Med* 314:131-2, 1986.
9. Schuurman H-J, Krone WJA, Broekhuizen R, Goudsmit J. Expression of RNA and antigens of human immunodeficiency virus type-1 (HIV-1) in lymph nodes from HIV-1 infected individuals. *Amer J Pathol* 1988,133:516-524.
10. Baroni CD, Pezzella F, Pezzella M, Macchi B, Vitolo D, Uccini S, Ruco LP. Expression of HIV in lymph node cells of LAS patients. Immunohistology, *in situ* hybridization, and identification of target cells. *Amer J Pathol* 1988,133:498-506.
11. Schnittman SM, Psallidopoulos MC, Lane HC, Thompson L, Baseler M, Massari F, Fox CH, Salzman NP, Fauci AS. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science* 245:305-308, 1989.
12. Harper ME, Marselle IM, Gallo RC, Wong-Staal FW. Detection of lymphocytes expressing human T-lymphotrophic virus type III in lymph nodes and peripheral blood from infected individuals by *in situ* hybridization. *Proc Natl Acad Sci USA* 83:772-776, 1986.